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## DO PARTICLES TRANSLOCATE FROM THE VAGINA TO THE OVIDUCTS AND BEYOND?

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**Abstract**—To investigate whether particles deposited in the vagina translocate to the oviducts, 0.3 ml of a 4% bone black suspension was deposited in the posterior vaginal fornix of each of five cynomolgus monkeys (*Macaca fascicularis*) during their mid-menstrual cycle. Simultaneously, each animal received 10 units of oxytocin by intramuscular injection. The oviducts of three animals were removed 1 hr after administration of the bone black, while those of the remaining two animals were removed 72 hr after dosing. The removed oviducts were flushed with Hank's solution and then with collagenase solution. The solutions were collected in clean vials and filtered. The filters were examined for bone black particles by light microscopy, as were filters through which solution blanks (negative controls) had been passed. Particles resembling bone black were found on all filters. There were no appreciable differences in the numbers or shape of these particles between the solution-blank filters and the oviduct-flush filters. The particles on both the solution-blank filters and on the oviduct-flush filters probably originated from environmental contamination by ubiquitous carbon particles. While these results suggested that no translocation took place, translocation could not be ruled out with certainty in the absence of quantitative analyses. A more definitive pilot study was then conducted with two dosed monkeys and one control, using talc labelled by neutron activation to circumvent the problem of environmental contamination.  $\gamma$ -Ray analysis of tissue and peritoneal lavage samples for the radionuclides  $^{46}\text{Sc}$ ,  $^{59}\text{Fe}$  and  $^{60}\text{Co}$  indicated that no measurable quantities (i.e.  $> 0.5 \mu\text{g}$ ) of talc translocated from the deposition site in the vagina to the uterine cavity and beyond.

### INTRODUCTION

Egli & Newton (1961) deposited carbon black (bone black) particles, suspended in 30% dextran, in the posterior vaginal fornix in three anaesthetized women who were about to undergo hysterectomy at the approximate time of ovulation. Concurrent with the carbon black dosing, oxytocin was injected intramuscularly. In two of the three women, "a few" (G. E. Egli, personal communication, 1983) carbon black particles were observed in the liquid with which the oviducts were flushed half an hour after carbon black deposition. These findings were generally taken to indicate the translocation of particulate matter from the vagina to the oviducts and, possibly, to the ovaries.

Interest in this subject led to the desire to investigate this phenomenon further in a suitable animal model. Following the procedures of Egli & Newton (1961) as closely as practical, we attempted to reproduce their results in the cynomolgus monkey. The experiments were conducted following the US Food and Drug Administration's Good Laboratory Practice regulations for non-clinical laboratory studies.

### EXPERIMENTAL

#### Bone black deposition study

**Animals.** Female cynomolgus monkeys (*Macaca fascicularis*; exbreeders, weighing 2.5–4.0 kg), were obtained from the Medical Lake Field Station of the

Regional Primate Research Center at the University of Washington. The animals were housed individually in primate cages (24 in.  $\times$  24 in.  $\times$  32.5 in.) with free access to water. Each animal was fed 12–18 regular Purina Monkey Chow biscuits (Ralston Purina Co., Inc., St Louis, MO) once a day, supplemented by a twelfth of an apple and a twelfth of an orange.

**Suspensions and solutions.** Suspensions of 4% bone black (Arthur H. Thomas, Philadelphia, PA) in 30% dextran (clinical grade; Sigma Chemical Co., St Louis, MO) solution, and of 4% bone black in physiological saline solution containing 1% carboxymethyl cellulose (CMC; sodium salt, low viscosity; Sigma) were prepared by registered pharmacists at the Department of Pharmacy Services of the Oregon Health Sciences University. The suspension of bone black in saline solution containing CMC was used in addition to the procedures of Egli & Newton (1961). The rationale for using this additional suspension was that particles in the vagina are not usually trapped in viscous seminal fluid, and the objective of our study was to determine whether bone black particles translocate from the vagina to the oviducts under the most favourable naturally occurring conditions. Hank's solution for the first flushing of the oviducts (see below) was prepared and filtered through a 0.22- $\mu\text{m}$  Millipore filter into empty sterile vials and stored under refrigeration until used. Collagenase (United States Biochemical Co., Cleveland, OH) solutions for the second rinsing of the oviducts (see below) were freshly prepared

before the kills and passed through a 0.22- $\mu$ m Millipore filter.

*Experimental procedures.* Five monkeys were selected, based on their relatively synchronous menstrual cycles which facilitated the deposition of bone black in mid-cycle in all five monkeys on the same day. Menstruation was determined by visual inspection of the stainless-steel collecting pans under their cages for menstrual blood. Mid-cycle dosing was done to simulate the procedures used by Egli & Newton (1961) and to enhance the chances of particle translocation from the vagina to the oviducts.

For dosing, the five monkeys were placed on their backs and restrained by taping their hands and tails to a plywood restraining cross. The pelvis was elevated at an angle of 15° and the legs were held with the knees bent close to the chest by an assistant. A nasal speculum was inserted into the vagina and opened to expose the cervix. Each of four animals received 0.3 ml of 4% bone black suspended in 30% dextran solution, and one animal received 4% bone black in 0.3 ml saline containing 1% CMC. The bone black suspension was deposited in the posterior fornix of the vagina, using a 1.0-ml Tuberculin syringe with a stainless-steel animal feeding needle (CVD 18 gauge  $\times$  1.5 in.; Popper & Sons, Inc., New Hyde Park, NY). Ten units of oxytocin (oxytocin injection, U.S.P. synthetic; Invenex, Division of Dexter Corp., Chagrin Falls, OH) were injected intramuscularly at the same time as the bone black was deposited. Following the deposition of bone black, the animals were maintained in the restrained position as described above for approximately 20 min and then returned to their cages.

Approximately 1 hr following the deposition of bone black, two of the monkeys that received bone black in dextran and the monkey that received bone black in saline with CMC were anaesthetized with 75 mg ketamine hydrochloride. The two remaining monkeys were similarly anaesthetized 72 hr after the deposition of bone black. With the site of the abdominal incision shaved before surgery, the abdominal cavity was opened immediately. A new set of sterile instruments was used for each animal. The oviducts were clamped at the isthmus and removed. The remainder of the reproductive tract was then collected and preserved in formalin for future reference. A 3-ml syringe with a 20 gauge needle inserted into a PV2 or PV3 catheter, was used to flush the oviducts. The syringe was filled with Hank's solution and the catheter was inserted into the oviduct through the fimbrial ostium. The solution was flushed through the duct and collected in a clean 60  $\times$  15 mm petri dish. After collection, the solution was transferred into a screw-cap polyethylene vial. The flushing solution from both oviducts of one animal was collected in one vial. Before use the vials were rinsed with deionized water and air-dried. The oviducts were then flushed with 0.05% calcium-free collagenase solution which was collected in a different clean screw-cap vial. The purpose of flushing the oviducts with collagenase solution (not done by Egli & Newton, 1961) was to remove and collect particles adhering to the surface of the mucous membrane of the oviducts from which they might not be dislodged by flushing with Hank's solution.

Similarly, all of the above mentioned solutions, without bone black, were injected into separate petri dishes and transferred into screw-cap vials as negative controls (blanks). An additional vial was injected with a suspension of bone black to serve as a positive control.

*Sample processing.* After collection the sample were frozen until required. Before processing, the samples were thawed and immediately filtered under vacuum, using Millipore type HAWP04700 membrane filters (0.45  $\mu$ m) mounted in standard Millipore filter holders. The filtration apparatus was covered with a lint-free nylon cloth during filtration to prevent contamination of the sample with airborne dust. After filtering an oviduct flush sample, the sample vial was rinsed three times with approximately 1 ml of deionized water and the rinses were passed through the same filter as the sample. Our preliminary studies had shown that the control samples were too viscous to filter without dilution. Therefore the rinse water was added to the sample on the filter. The sample number was marked in ink on the border of each filter before it was removed from the filter holder, and then the filter was placed on a paper disk in a covered petri dish for later examination.

Approximately 5 ml each of the Hank's and collagenase solutions, and 100 ml of the deionized water used to rinse the sample vials, were also filtered and examined for the presence of particles resembling bone black. A new filter disk was examined to determine whether the filters were free of particulates prior to use. Finally, a dilute suspension of bone black was prepared in deionized water and filtered to provide a bone black reference for comparison with the oviduct flush filters and the solution blank (control) filters.

*Examination of filters.* Each filter was carefully examined under the light microscope ( $\times$  100) for bone black particles. The filter was repeatedly scanned, and various different fields on the filter were observed. Points of interest were examined under higher magnification ( $\times$  450). A quantitative evaluation was neither planned nor attempted.

#### Talc deposition study

*Neutron activation and preparation of talc.* Neutron irradiation and homogenization of a purified cosmetic talc blend were similar to previously described procedures (Wehner, Wilkerson, Cannon *et al.* 1977; Wehner, Wilkerson, Mahaffey *et al.* 1980; Wehner, Wilkerson & Stevens, 1984). The talc samples were exposed to a calculated neutron fluence of  $1.2 \times 10^{17}$  n/cm<sup>2</sup>. Activities induced in the talc included <sup>141</sup>Ce (0.018  $\pm$  3.2%), <sup>60</sup>Co (0.297  $\pm$  0.9%), <sup>58</sup>Co (0.0839  $\pm$  1.0%), <sup>51</sup>Cr (2.29  $\pm$  0.9%), <sup>59</sup>Fe (0.617  $\pm$  0.7%), <sup>177</sup>Lu (0.093  $\pm$  9.3%), <sup>54</sup>Mn (0.026  $\pm$  2.1%), <sup>124</sup>Sb (0.0039  $\pm$  11.7%), <sup>46</sup>Sc (0.316  $\pm$  0.7%), <sup>169</sup>Yb (0.010  $\pm$  12%) and <sup>65</sup>Zn (0.015  $\pm$  6.9%). The numbers in parenthesis give the radionuclide activities in dpm/ $\mu$ g talc at the time of analysis of the tissue and lavage samples. The percentage values listed are the relative percent standard deviation determined in the analysis.

*Experimental procedures.* Similar dosing procedures to those described for the bone black study

ove mentioned solutions, injected into separate petri into screw-cap vials as.

An additional vial was of bone black to serve as a

collection the samples i. Before processing, the immediately filtered under type HAWP04700 mem- mated in standard Millipore n apparatus was covered loth during filtration to the sample with airborne iduct flush sample, the three times with approxi- water and the rinses were filter as the sample. Our shown that the control to filter without dilution. was added to the sample on ber was marked in ink on before it was removed from the filter was placed on red petri dish for later

ach of the Hank's and 1 100 ml of the deionized triple vials, were also filtered essence of particles resem- filter disk was examined to rs were free of particulates dilute suspension of bone nized water and filtered to rence for comparison with and the solution blank

Each filter was carefully t microscope ( $\times 100$ ) for he filter was repeatedly rent fields on the filter were test were examined under 450). A quantitative eval- nor attempted.

*Preparation of talc.* Neutron activation of a purified cosmetic to previously described erson, Cannon *et al.* 1977; affey *et al.* 1980; Wehner, 14). The talc samples were atron fluence of  $1.2 \times 10^{17}$  in the talc included  $^{141}\text{Ce}$   $7 \pm 0.9\%$ ,  $^{59}\text{Co}$  (0.0839  $\pm$  %),  $^{59}\text{Fe}$  (0.617  $\pm$  0.7%),  $^{55}\text{Mn}$  (0.026  $\pm$  2.1%),  $^{122}\text{Sb}$  (0.316  $\pm$  0.7%),  $^{169}\text{Yb}$  Zn (0.015  $\pm$  6.9%). The ve the radionuclide activi- time of analysis of the es. The percentage values ercent standard deviation

Similar dosing pro- for the bone black study

were used. Each of two monkeys received approximately 125 mg neutron-activated talc, suspended in 0.3 ml deionized water containing 1% CMC.

*Sacrifice and tissue collection.* Three days after the talc deposition, the animals were anaesthetized by intramuscular injection of 100 mg (1 ml) ketamine hydrochloride. The abdominal area was shaved. A peritoneal lavage was performed to recover talc particles that might have translocated to the peritoneal cavity. This was carried out by injecting approximately 135 ml of physiological saline solution into the peritoneal cavity, followed by brief gentle massage to distribute the lavage fluid and wash off any talc particles that might have adhered to the serous membranes of the peritoneal cavity. The peritoneal cavity was then opened by incision and the lavage fluid collected by aspiration with a calibrated syringe and transferred into a polyethylene vial for  $\gamma$ -ray analysis. The lavage was repeated once through the abdominal incision of the first of the two animals to determine whether, in the case of talc translocation from the vagina into the abdominal cavity, there would be a difference in  $\gamma$ -ray counts between the one-lavage and the two-lavage samples.

Precautions to avoid contamination and cross-contamination of samples included the use of clean instruments for each sample to be collected and starting with the collection of samples least likely to contain translocated talc, i.e. the ovaries. Right and left ovaries were collected in one polyethylene vial for  $\gamma$ -ray analysis. Right and left oviducts were similarly collected and sectioned into three parts of approximately equal length and placed in three vials for  $\gamma$ -ray analysis, followed by collection of the body of the uterus. Because deposition of talc in the vaginal fornix might also result in the direct mechanical deposition (rather than physiological translocation) of talc in the uterine cervix, the cervix of the uterus was dissected from the body and analysed together with the vagina. The animals were then humanely killed by iv injection of a barbiturate-based solution. One untreated control animal was processed first, following the same procedures, to

provide background levels of the radionuclides in the tissues of interest.

*Gamma-ray analysis.* The samples were placed under an infra-red heat lamp to evaporate excess moisture. The lavage fluid samples were evaporated until approximately 2.5 ml of liquid remained. The samples were then counted using multidimensional and coincidence-noncoincidence detector systems. The samples were counted for a minimum of 1000 min on either system.

A sample of National Bureau of Standards 4276-B liquid standard was used to calibrate spikes of  $^{59}\text{Fe}$ ,  $^{60}\text{Co}$  and  $^{46}\text{Sc}$ . These calibrated spikes were then used with both types of analyser systems to determine the appropriate calibration factors for the counting geometries used.

## RESULTS

### *Bone black deposition study*

Results of the filter examinations are summarized in Table 1. Particles resembling bone black were found on all filters through which the solution blanks or the oviduct flushing solutions had been passed. They ranged in numbers from very few to occasional on all filters. No distinct differences in numbers or shape of these particles were apparent (1) among the Hank's solution blank, the collagenase solution blank and the physiological saline + CMC blank, (2) between the Hank's oviduct flush solution and collagenase oviduct flush solution from each animal, (3) between the oviduct flush solutions from the animals dosed with 4% bone black suspended in 30% dextran and the oviduct flush from the animal dosed with 4% bone black suspended in physiological saline containing 1% CMC, (4) between oviduct flush solutions from the three animals sacrificed 1 hr after dosing and the two animals sacrificed 72 hr after dosing, and (5) between the blank solutions and the oviduct flush solutions. The new filter blank, removed from its container immediately before examination under the microscope, was the only sample on which no bone black particles were found.

Table 1. Results of microscopic examination of filters in bone black deposition study

Treatment	Animal no.	Presence of particles*	
		First flush: Hank's solution	Second flush: Collagenase solution
4% Bone black in 30% dextran	80-232	+	+
4% Bone black in 30% dextran	81-056	+	+
4% Bone black in 30% dextran	81-138	+	+
4% Bone black in 30% dextran	81-158	+	+
4% Bone black in saline + 1% CMC	81-142	+	+
Controls			
Clean filter		—	
Hank's solution blank		+	
Collagenase solution blank		+	
30% dextran		+	
Saline + 1% CMC		+	
4% bone black in 30% dextran		+++	

CMC = Carboxymethyl cellulose

\*Key: (—) no particles found, (+) very few to occasional particles found, (++) very many particles found.

Since these results were inconclusive, a very similar study was conducted on a pilot basis in two monkeys using neutron-activated talc to obtain more definitive and quantitative data and to circumvent the problem of environmental contamination caused by apparently ubiquitous bone black particles.

#### Talc deposition study

Data generated from both analyser systems were compared and compiled in Table 2. The data in Table 2 indicate that there was no measurable translocation of activated talc from site of deposition in the posterior vaginal fornix to the uterine cavity, oviducts, ovaries or peritoneal cavity. Although sample 3c from monkey 81-090 and sample 3b from monkey 79-276 suggest detectable amounts of  $^{60}\text{Co}$ , the implication that talc might have translocated to these sections of the oviducts is not borne out by the  $^{46}\text{Sc}$  or the  $^{59}\text{Fe}$  data. Furthermore, if the talc had translocated to these sections of the oviducts, it would be reasonable to expect also some talc deposition in tissues more proximal to the original deposition site (i.e. in sample 4 from monkey 81-090, and samples 3c and 4 from monkey 79-276).

A  $2\sigma$  detection limit can be determined for the detection of  $^{46}\text{Sc}$  and  $^{60}\text{Co}$  by the multidimensional detectors as well as for  $^{59}\text{Fe}$ , which was determined by the coincidence-noncoincidence detector systems. These detection limits were found to be 0.16, 0.16 and

2.0 dpm/sample, respectively. Alternatively, this may be expressed as being able to detect 0.51, 0.54 or 3.2  $\mu\text{g}$  of talc, based on  $^{46}\text{Sc}$ ,  $^{60}\text{Co}$  or  $^{59}\text{Fe}$  data, respectively, or (based on the more sensitive  $^{46}\text{Sc}$  and  $^{60}\text{Co}$  data) approximately 1/245,000 to 1/230,000 of the estimated initial deposition of 125  $\mu\text{g}$  in the vagina. Of this original deposition, approximately 2.3 mg and 300  $\mu\text{g}$  were found in the vaginas of the two monkeys 3 days after dosing.

#### DISCUSSION

Experimental observations in animals suggest that uterine contractions during copulation aid in transporting spermatozoa from the vagina to the oviducts, and it has been speculated that oxytocin might be a mediator of this process (Egli & Newton, 1961). However, attempts to improve sperm transport and fertility by injection of drugs—including oxytocin and relaxin—have, in general, not been successful (Salamon & Lightfoot, 1970; Gustafsson & Memon, 1978). Nevertheless, oxytocin was administered to simulate in the animal model as closely as practical the experimental conditions described by Egli & Newton (1961).

Egli & Newton (1961) anaesthetized their three patients in preparation for the hysterectomies that followed their bone black deposition experiment. We did not anaesthetize the monkeys in the present

study; this was  $\pm$  of the anaestheti

The validity animal model sc lished by inferer the reproductive cynomolgus mo more closely tha tory animal. Th approximately 2-7 days. Its passage is obv of the species. cynomolgus mo ticles translocat beyond appears female herself.

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Table 2. Radionuclide activities in lavage and tissue samples, and corresponding quantities of talc

Animal no.	Sample no.*	Radionuclide activity (dpm)		
		$^{46}\text{Sc}$	$^{59}\text{Fe}$	$^{60}\text{Co}$
81-140 (control)	1	<0.1	<1	<0.1
	2	<0.1	<1	<0.1
	3a	<0.1	<1	<0.1
	3b	<0.1	<0.7	<0.1
	3c	<0.1	<0.9	<0.1
	4	<0.08	<2	<0.08
	5	<0.1	<0.9	<0.09
81-090	1	<0.08	<2	<0.08
	2	<0.1	<1	<0.09
	3a	<0.1	<0.7	<0.1
	3b	<0.1	<0.5	<0.4
	3c	<0.1	<0.9	$0.44 \pm 0.10$
	4	<0.7	<1	<0.08
	5	$690 \pm 10$	$1390 \pm 20$	$710 \pm 10$
79-276	5†	c. 2.18 mg talc	c. 2.25 mg talc	c. 2.39 mg talc
		Mean = 2.27 mg talc		
	1	<0.1	<2	<0.1
	2	<0.09	<0.9	<0.09
	3a	<0.1	<1	<0.1
	3b	<0.1	<0.8	$0.63 \pm 0.12$
	3c	<0.1	<0.6	<0.1
	4	<0.7	<1.4	<0.08
	5	$87 \pm 2$	$264 \pm 8$	$85 \pm 2$
	5†	c. 275 $\mu\text{g}$ talc	c. 399 $\mu\text{g}$ talc	c. 286 $\mu\text{g}$ talc
		Mean = 320 $\mu\text{g}$ talc		

\*Sample numbers refer to the following: 1 = peritoneal lavage; 2 = ovaries; 3a = lateral thirds of oviducts; 3b = centre thirds of oviducts; 3c = mesial thirds of oviducts; 4 = body of uterus; 5 = vulva, vagina and cervix.

†Based on the following values for dpm/ $\mu\text{g}$  talc:  $^{46}\text{Sc} = 0.316$ ;  $^{59}\text{Fe} = 0.617$ ;  $^{60}\text{Co} = 0.297$ .

Values after the “ $\pm$ ” symbols are standard deviations produced by the counting statistics.

ly. Alternatively, this may be to detect 0.51, 0.54 or  $^{46}\text{Sc}$ ,  $^{60}\text{Co}$  or  $^{59}\text{Fe}$  data, ie more sensitive  $^{46}\text{Sc}$  and 1/245,000 to 1/230,000 of sition of 125 mg in the deposition, approximately und in the vaginas of the osing.

## SION

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study; this was to avoid possible attenuating effects of the anaesthetic on uterine contractions.

The validity of the cynomolgus monkey as an animal model for the human female may be established by inference. The anatomy (including that of the reproductive tract) and physiology of the cynomolgus monkey resemble that of *Homo sapiens* more closely than any other readily available laboratory animal. This monkey has an oestrous cycle of approximately 28 days, with menstruation lasting 2-7 days. Its relatively non-convoluted cervical passage is obviously patent, at least for spermatozoa of the species. Therefore the selection of the cynomolgus monkey for determining whether particles translocate from the vagina to the oviducts and beyond appears to be the best choice, save the human female herself.

Microscopic examination of the filters from the bone black deposition study gave the impression that there were no apparent differences in the number of bone black particles between the solution blanks and the oviduct rinse solutions. This would suggest that no translocation of bone black particles from the vagina to the oviducts took place. However, since no quantitative analyses were planned or conducted, translocation of particles could not be ruled out with certainty, based on the bone black study alone.

If no solution blanks had been examined, the conclusion from the results of this study would have been that bone black does, indeed, translocate from the vagina to the oviducts. Egli & Newton (1961) did not examine blanks (G. E. Egli, personal communication, 1983). This, in the light of our findings, suggests the possibility of a 'false positive' in their interpretation of their observations.

The presence of bone black particles in the solution blanks in this study raises the question of their origin. Two potential causes could be: (1) accidental contamination in the laboratory during sample preparation, and (2) background levels of carbon particles in reagents and elsewhere due to the ubiquity of carbon as a universal air pollutant. The first possibility can be ruled out with a high degree of confidence because (a) the flushing solutions were prepared on a different day than the carbon suspension and (b) the awareness of potential contamination problems led to great care in preparing the solutions and filtering the samples. This suggests that the universal air pollution with carbon particles may have been responsible for the presence of carbon particles in the oviduct flushing solutions as well as in the solution blanks. This possibility is supported by the observation that an estimated 6 million tons of fly ash are being dispersed annually into the atmosphere from the combustion of coal in the United States (EPA, 1973; U.S. Bureau of Mines, 1970), making carbon one of the most prevalent and ubiquitous air pollutants.

Neutron activation appears to be the most suitable technique for a definitive study because (1) it is extremely sensitive, allowing the monitoring of deposition, translocation and clearance of minute quantities of material in the tissues of laboratory animals, (2) it circumvents the problem of environmental contamination, and (3) monitoring for more than one radionuclide provides information on

whether the radionuclides represent particulate matter or just elements leached from the particles (Wehner *et al.* 1977, 1980 & 1984; Wehner & Wilkerson, 1981). Use and subsequent leaching of only one radioactive label, such as  $^{99}\text{Tc}$  from human albumin microspheres (Venter & Iturralde, 1979), can result in misleading data (Bolles, Kubiatowicz, Evans *et al.* 1973; Subramanian, Rhodes, Cooper & Sodd, 1975). The results of our more definitive pilot study with labelled talc support the findings of our bone black study by indicating that, under our experimental conditions, no measurable quantities (i.e.  $> 0.5 \mu\text{g}$ ) of 'insoluble' particles translocate from the vagina to the oviducts and beyond.

Using the same technique, we are now investigating in an expanded study with more animals whether multiple talc applications, covering at least one complete menstrual cycle, will result in talc translocation from the vagina through the cervical canal and the uterine cavity to the oviducts and beyond.

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**Abstract**—The c point mutation hamster cells in bone marrow o responses were

### Introduction

In modern agricultu increasingly for plant chemical agents shou without having harr ganism. Among the ef be a number of pot genic compounds (V 1983).

Thiram, a fungicid class, has been four reverse gene mutati strains of *Salmonell* TA1538 and TA98), f 12 and direct gene n (Zdziennicka, Zieleni Zdziennicka, Zielenśk: also induces abnorm heterozygous diploid Johnson, 1981). The some other dithioc accelerators in the S shown in Ames t Rannug, Ramel & there are some confl the one hand, the cy noted in bone-marr (Pilinskaya, Kurinni human embryonic fi but on the other t induction of micron ICR mice after oral tolerated dose of thir

The aim of this v thiram to induce hypoxanthine-guanine (HGPRT) locus in C to increase the inci marrow polychromat

### Experimental

#### Test material

Thiram (TMTD; grade dry powder co